Claims

1. Apparatus for culturing cells, comprising:

a vessel for holding liquid cell culture media,

a matrix assembly mounted in the vessel for movement in the media, said matrix assembly including a support and a plurality of three-dimensional porous matrix members carried by the support for movement therewith, and

a drive member operatively coupled to the support for moving it with the matrix members through the media.

2. Apparatus as defined in claim 1, wherein the support includes a shaft, an outwardly extending member from and connected to the shaft, and a holder for carrying at least one three-dimensional porous matrix member and mounted on the outwardly extending member.

3. Apparatus as defined in claim 2, wherein the holder is removably attached to the outwardly extending member.

4. Apparatus as defined in claim 2, wherein the outwardly extending member carries a plurality of holders.

5. Apparatus as defined in claim 3, wherein the holder carries a plurality of three-dimensional porous matrix members.

6. Apparatus as defined in claim 4, wherein at least one of the holders carries a plurality of three-dimensional porous matrix members.

7. Apparatus as defined in claim 2, wherein a plurality of outwardly extending members extend radially outwardly from the shaft, each outwardly extending member carrying at least one holder.

8. Apparatus as defined in claim 3, wherein the holder is detachably connected to the outwardly extending member.

9. Apparatus as defined in claim 3, wherein the holder is mechanically coupled to the outwardly extending member for releasably retaining the holder on the outwardly extending member.

10. Apparatus as defined in claim 3, wherein the holder is made of rigid plastic material and includes a U-shaped frame having an open and a closed end with a pair of opposed sides, said frame being attached at its closed end to the outwardly extending member, said sides having receptacles for receiving at least one three-dimensional porous matrix member and releasably holding the three-dimensional porous matrix member in place on the holder.

11. Apparatus as defined in claim 10, wherein the shaft is supported in the vessel for rotation about the shaft axis.

12. Apparatus as defined in claim 11, wherein the shaft is supported vertically in the vessel and supported therein from its top end.

13. Apparatus as defined in claim 12, wherein the outwardly extending member is disposed in the vicinity of the lower end of the shaft.

14. Apparatus for culturing cells, comprising:

a relatively rigid vessel for holding liquid cell culture media having an opening for providing access to its interior and a cover for the opening,

a shaft disposed in the vessel and supported for rotation in the vessel by the cover,

an outwardly extending member attached to the shaft extending outwardly from the axis of rotation of the shaft for rotation therewith,

a plurality of holders attached to the outwardly extending member, and

at least one three-dimensional porous matrix member carried by the holders for rotation with the shaft in the media.

15. Apparatus for culturing cells, comprising:

a relatively rigid vessel for holding liquid cell culture media having an opening for providing access to its interior and a cover for the opening,

a shaft supported in the vessel by the cover,

an outwardly extending member attached to the shaft extending outwardly from the axis of rotation of the shaft for rotation therewith,

a plurality of holders attached to the outwardly extending member, and

at least one three-dimensional porous matrix member carried by the holders for rotation with the outwardly extending member in the media.

16. Apparatus as described in claim 14, wherein a motor drive is disposed outside the vessel and magnetically coupled to the shaft for rotating the shaft in media in the vessel.

17. Apparatus as described in claim 15, wherein a motor drive is disposed outside the vessel and magnetically coupled to the member for rotating the holder in media in the vessel.

18. Apparatus as described in claim 16, wherein the holder comprises a pair of substantially parallel arms connected together at one end by a base arm and having an open end at the other, a mounting device connected to the base arm for mounting the holder to a support, and a groove in each of the arms generally facing one another to engage the at least one three-dimensional porous matrix member.

19. Apparatus as described in claim 18, wherein a plurality of outwardly extending members extend outwardly from the shaft, each of said members having at least one station for connection to the base arm of the holder.

20. Apparatus as described in claim 19, wherein the at least one station includes a recess, and the base of the holder is connected to a mounting device that fits into the recess to mechanically keep the holder in place on the outwardly extending member.

21. Apparatus as described in claim 18, wherein the three-dimensional porous matrix member is circular and has an edge that engages the arms.

22. Apparatus as described in claim 18, wherein the three-dimensional porous matrix member is rectangular and has an edge that engages the arms.

23. A matrix assembly for culturing cells, comprising:

a generally U-shaped holder having a pair of substantially parallel arms connected together at one end by a base arm and having an open end at the other,

a mounting device joined to the base arm for mounting the holder to a support,

a groove in each of the arms generally facing one another, and

at least one three-dimensional porous matrix member having its periphery removably disposed in the groove of each arm.

24. A matrix assembly as defined in claim 23, wherein a plurality of separate three-dimensional porous matrix members are mounted on the holder with their peripheries disposed in the groove.

25. A matrix assembly as defined in claim 23, wherein the three-dimensional porous matrix member has a circular shape.

26. A matrix assembly as defined in claim 24, wherein the three-dimensional porous matrix member has a rectangular shape.

27. A matrix assembly as defined in claim 23, wherein the groove in each of the arms extends to the open end of the holder for enabling the periphery of the three-dimensional porous matrix member to be slipped into and between the grooves from the open end.

28. A matrix assembly, comprising:

a generally U-shaped holder having a pair of spaced apart coplanar arms connected together at one end by a base arm and having an open end at the other,

at least one three-dimensional porous matrix member carried by the arms and detachably connected thereto, said three-dimensional porous matrix member being disposed between and in the plane of the arms,

a mounting device attached to the base arm for mounting the holder to a support, and

a closure-cap detachably mounted to the open end of the arms for preventing the three-dimensional porous matrix member being withdrawn from between the arms, said closure-cap having a handle for stripping the closure-cap from the holder and for carrying the assembly without touching the three-dimensional porous matrix member.

29. A matrix assembly as described in claim 28, wherein the closure-cap has an end wall for spanning the open end space between the arms and a pair of legs for engaging the sides of the arms, and connectors on the arms and the legs for engaging one another to releasably hold the closure-cap in place on the holder.

30. A matrix assembly as described in claim 29, wherein the legs are of unequal length and the handle is disposed on the side of the closure nearer the longer leg.

31. A matrix assembly as described in claim 29, wherein the connectors are detents and recesses disposed on arms and the ends of the legs.

32. A matrix assembly as described in claim 31, wherein the connectors are disposed on the ends of the legs.

33. A matrix assembly as described in claim 29, wherein the assembly includes a plurality of three-dimensional porous matrix members disposed between the arms.

34. A matrix assembly as defined in claim 23, further comprising a support-wheel, said support-wheel comprising:

a hub,

at least one arm extending outwardly from the hub, and

at least one station on the at least one arm extending outwardly from the hub for receiving the mounting device of the holder.

35. A matrix assembly and support combination as defined in claim 34, wherein the holder has a plurality of outwardly extending arms as described, and each arm carries at least one matrix assembly.

36. A matrix assembly as defined in claim 34, wherein the mounting device of the holder is a snap fastener for engaging the at least one station.

37. A matrix assembly as defined in claim 34, wherein the mounting device of the holder is a snap fastener for retaining the holder on the support.

38. A support and matrix assembly for culturing cells, comprising:

a shaft for disposition in vessel containing liquid cell culture media,

a support-wheel mounted on the shaft and having a plurality of arms extending outwardly away from the shaft, each of said arms having at least one station for carrying a matrix assembly,

a plurality of matrix assemblies mounted on each of the stations, each assembly including a U-shaped holder having a pair of generally parallel and spaced apart arms connected together at one end by a base arm and having an open end at the other,

a mounting device attached to the base arm for mounting the holder to the station on the arm of the support-wheel,

a groove on each of the arms of the holder facing one another for supporting three-dimensional porous matrix members between and generally in the plane of the arms, and

a closure-cap for each of the holders for retaining the three-dimensional porous matrix member, between the arms.

39. A support and matrix assembly as defined in claim 38, wherein each holder carries a plurality of three-dimensional porous matrix members.

40. A support and matrix assembly as defined in claim 39, wherein the support-wheel has eight arms.

41. A support and matrix assembly as defined in claim 40, wherein each arm of the support-wheel has three stations.

42. A support and matrix assembly as defined in claim 38, wherein each holder carries at least four three-dimensional porous matrix members.

43. A matrix assembly as defined in claim 23, wherein the holder is made of plastic.

44. A matrix assembly as defined in claim 23, wherein the holder is disposable.

45. A matrix assembly as defined in claim 38, wherein the holder is made of plastic.

46. A matrix assembly as defined in claim 38, wherein the holder is disposable.

47. A matrix assembly as defined in claim 34, wherein the holder and support-wheel are made of plastic.

48. A matrix assembly as defined in claim 34, wherein the holder and support-wheel are disposable.

49. A matrix cartridge for culturing cells, comprising:

a generally U-shaped holder having a pair of spaced apart coplanar arms connected together at one end by a base arm and having an open end at the other,

at least one three-dimensional porous matrix member carried by the arms and detachably connected thereto, said three-dimensional porous matrix member being disposed between and in the plane of the arms,

a mounting device attached to the base arm for mounting the holder to a support,

a closure-cap detachably mounted to the open end of the arms for preventing the three-dimensional porous matrix member being withdrawn from between the arms, said closure-cap having a handle for stripping the closure-cap from the holder and for carrying the assembly without touching the three-dimensional porous matrix member, and

a container having a well for an assembled holder, closure-cap and three-dimensional porous matrix member, said container having a cover for sealing the well with the holder, closure-cap and three-dimensional porous matrix member.

50. A matrix cartridge as defined in claim 49, wherein the well shape conforms to the shape of the assembled holder, closure-cap and three-dimensional porous matrix member.

51. A matrix cartridge as defined in claim 50, wherein the well supports the assembled holder, closure-cap and three-dimensional porous matrix member so that the matrix member does not physically engage the well or cover.

52. A cartridge as defined in claim 51, wherein the well has a shoulder in the periphery that supports the holder away from the well bottom so that the three-dimensional porous matrix member does not engage said bottom.

53. Apparatus for culturing cells, comprising:

a vessel for holding liquid cell culture media,

a matrix assembly mounted in the vessel for movement in the media, said matrix assembly including a support and at least one three-dimensional porous matrix member carried by the support for movement therewith, and

drive means operatively coupled to the support for moving it with the matrix members through the media.

54. Apparatus as defined in claim 53, wherein the support includes a shaft means, means extending away form the shaft means, and means for carrying at least one three-dimensional porous matrix member and mounted on the means extending away form the shaft means.

55. Apparatus for culturing cells, comprising:

a relatively rigid vessel for holding liquid cell culture media having an opening for providing access to its interior and a cover for the opening,

first means disposed in the vessel and supported in the vessel by the cover,

an outwardly extending member attached to the first means and rotatable in the vessel,

a plurality of holders attached to the outwardly extending member, and three-dimensional porous matrix members carried by the holders, and

means for moving the outwardly extending member with the holders in the media.

56. A matrix assembly, comprising:

a holder having means for mounting the holder to a support,

engaging means on the holder for carrying a three-dimensional porous matrix member, and

at least one three-dimensional porous matrix member attached to the holder by the engaging means.

57. Apparatus as defined in claims 1-56, wherein the three-dimensional porous matrix member is an open cell porous matrix member having a percent open space of at least 75%.

58. Apparatus as defined in claim 57, wherein the three-dimensional porous matrix member has pores defined by interconnecting ligaments having a diameter at midpoint, on average, of less than 150μm.

59. Apparatus as defined in claim 57, wherein the three-dimensional porous matrix member is a metal-coated reticulated open cell foam of carbon containing material.

60. Apparatus as defined in claim 59, wherein the metal is selected from the group consisting of tantalum, titanium, platinum, niobium, hafnium, tungsten, and combinations thereof, wherein said metal is coated with a biological agent selected from the group consisting of collagens, fibronectins, laminins, integrins, angiogenic factors, anti-inflammatory factors, glycosaminoglycans, vitrogen, antibodies and fragments thereof, and combinations thereof.

61. Apparatus as defined in claim 59, wherein the metal is tantalum.

62. A method for *in vitro* culture of cells, comprising:

introducing an amount of cells into a three-dimensional porous matrix having interconnected pores of a pore size sufficient to permit the cells to grow throughout the matrix,

culturing the cells under conditions sufficient to allow the cells to adhere to the three-dimensional porous matrix, and

moving the three-dimensional porous matrix in a liquid cell culture medium under conditions sufficient to promote maintenance, expansion, or differentiation of the cells.

63. The method of claim 62, wherein the cells are selected from the group consisting of mammalian cells, animal cells, plant cells, eukaryotic cells, prokaryotic cells and genetically engineered cells.

64. The method of claim 62, wherein the cells are hematopoietic progenitor cells.

65. The method of claim 64, wherein the hematopoietic progenitor cells are cultured under conditions and for a time sufficient to increase the number of hematopoietic progenitor cells relative to the amount introduced into the three-dimensional porous matrix.

66. The method of claim 65, wherein the conditions exclude an exogenously added agent.

67. The method of claim 66, wherein the exogenously added agent is selected from the group consisting of a hematopoietic growth factor that promotes hematopoietic cell maintenance, expansion and/or differentiation, inoculated stromal cells and stromal cell conditioned medium.

68. The method of claim 64, further comprising:

before said introducing step, obtaining said hematopoietic progenitor cells from a blood product.

69. The method of claim 68, wherein said blood product is unfractionated bone marrow.

70. The method of claim 64, further comprising harvesting hematopoietic cells.

71. The method of claim 70, wherein said harvesting comprises a first harvesting after a first culturing period and at least one additional harvesting after at least one additional culturing period.

72. The method of claims 62-71, wherein the three-dimensional porous matrix is an open cell porous matrix having a percent open space of at least 75%.

73. The method of claim 72, wherein the three-dimensional porous matrix has pores defined by interconnecting ligaments having a diameter at midpoint, on average, of less than 150μm.

74. The method of claim 73, wherein the three-dimensional porous matrix is a metal-coated reticulated open cell foam of carbon containing material.

75. The method of claim 74, wherein the metal is selected from the group consisting of tantalum, titanium, platinum, niobium, hafnium, tungsten, and combinations thereof, wherein said metal is coated with a biological agent selected from the group consisting of collagens, fibronectins, laminins, integrins, angiogenic factors, anti-inflammatory factors,

glycosaminoglycans. vitrogen. antibodies and fragments thereof. and combinations thereof.

76. The method of claim 74, wherein the metal is tantalum.